# How to operate the BD FACSCanto flow cytometer

**Preface**

Dear colleague, the BD FACSCanto flow cytometer is use to operate, however, it is a delicate instrument for measuring fluorescence of single cells. This guide should help you operating the flow cytometer and leaving it such way that another colleague can use it another day. Therefore, please read these instructions carefully and apply them appropriately. Maintenance, long cleaning procedures, and calibration of the flow cytometer will be done by me or somebody appointed by me. If you have any questions regarding the flow cytometer or your experimental setup or when the flow cytometer is not working as it should, please contact me (j.m.mols@rug.nl or nr. 2107).
Good luck,

Maarten

**Introduction**

A flow cytometer is a device capable of measuring several parameters of a single cell. Typically a flow cytometer can measure: forward scatter (FSC, which is a measure for particle size), side scatter (SSC, which is a measure for particle complexity and density), and several fluorescence signals at different wavelengths (Fl-1 green, Fl-2, orange, Fl-3 red, Fl-4 far red). It can measure these parameters very fast. The FACSCanto can measure reliably up to approximately 12.000 events (cells, beads, particles) per second in a stream of fluid. How a flow cytometer does that is explained here:
http://probes.invitrogen.com/resources/education/tutorials/4Intro_Flow/player.html

Without software, hardware is useless and thus the flow cytometer is operated via a PC with special software: FACSDiva. You can find the icon of the program in the middle of the desktop. Do not use the FACSCanto software that is only used for calibration. There are several tutorials and manuals about the software placed in a folder called FACSDiva Manuals on the desktop.

**Flow cytometer startup**

The startup procedure takes about 7 minutes, so start this procedure about 7 minutes before you want to run your samples
-Press the green button on the left side of the flow cytometer, the green button should light up.
-Turn the computer on, password to start windows: **BDIS**.
-Start the program FACSDiva software, by clicking the icon in the middle of the desktop, press enter when the software is asking for a password (there's no password required).
-Click on **Instrument>Fluidics startup** and click on OK to start the automatic startup procedure.

-In the middle of the window the time is indicated for warming up the laser and there are four indicators. Three green indicators for FACSFlow, FACSClean, and ShutDownSolution (full = green, red = level too low, change fluids) and one black indicator for Waste (empty = black, red = full, empty Waste fluid tank)
The flow cytometer is no ready to run samples, start cleaning procedures, and for calibration.

**Running samples on the flow cytometer**

This is just a very short quick guide to help you run samples on the flow cytometer. For more information or details please consult the manuals in the folder on the desktop called FACSDiva Manuals.

-On the left of the main window of the FACSDiva software there are folders bearing people's names. To create your own personal folder, right click on "Administrator" (on the top, above all the folders). Click on "New folder" and a folder named "Folder_001" will be placed at the bottom of the list of folders.

-Right click on the new folder and click on "Rename". Type your name and press enter.

-Right click on your personal folder, click on "New experiment" and click on OK. You can also copy someone else's experiment or your own previous experiment (right click> copy on the experiment you like to copy) and subsequently paste it without data (right click> paste on your personal folder). Copying another experiment will copy the instrument settings and the worksheet, but you do not copy the data (unless you really want to).

-Right click on your experiment and click on "Rename" to give the experiment the name you desire.

-Click on "Instr Settings" just below your experiment. This opens the instrument settings in de "Inspector window" at the bottom in the middle of the screen. Here you can change the instrument settings for your entire experiment (Note: you can change the instrument settings per samples if you want to).

-On top of the computer there is a "Cytometer Setup Report" with the voltage settings indicated. FSC: forward scatter, SSC: side scatter, Fl-1=FITC: green fluorescence, Fl-2=PE: orange fluorescence, Fl-3=PerCP: red fluorescence, Fl-4=PerCP-Cy5.5: far red fluorescence, Fl-5= PE-Cy7, far red fluorescence excited by red laser, and Fl-6=APC, far far red fluorescence excited by red laser. If you don't need a certain parameter (for instance you only need Fl-1 because you're looking for GFP expression) you can select a parameter (Fl-2, Fl-3, Fl-4, Fl-5, Fl-6) and click on the "Delete" button. You should set the voltage settings of the remaining parameters (FSC, SSC, Fl-1) according the "Cytometer Setup Report" (indicated in yellow). **Check all "Log" and "A" boxes.** Note, you may want to change the voltage settings based upon the cells you are interested in.

-Click on the second tab in the inspector window called "Threshold". Set the FSC threshold at 200 and click on the "Add" button. Set the threshold for the appeared SSC also at 200. Make sure that both thresholds are in place by checking the **"And"** box.

-Click on the third icon from the left in the "Worksheet" window (on the right) and draw a rectangle on the panel below the icons. A dotplot will appear (FSC:X-axis, SSC:Y-axis). Select the dotplot and check the boxes indicating **"X axis" and "Y axis"** just below "Biexponential Display" in the Inspector window. You may want to

create other dotplots or histograms (5th icon from the left), you can change the X or Y axes by right clicking on the name of the axis (for instance FSC).
-Now you're set to run your samples. It is important to start with a **positive control** sample, so you can change the instrument settings if needed. Right click on you experiment and click on "New specimen". You can regard this item as a rack for your sample tubes and rename it by right click>Rename.
-In your rack (the specimen) there is one tube called "Tube_001", rename it by right click>Rename.
-To run your sample you need the "**Acquisition Dashboard**". If you don't see this window go to View>Acquisition Dashboard" or press Ctrl+Shft+C.
-Before running your samples click on the "Remove Tube" button at the Acquisition Dashboard, put the SIT (tube holder) all the way to the left and let it go (don't do this too gentle). The needle-like structure for sucking up your sample is being rinsed and the flow is discarded automatically.
-Take a tube with your sample, vortex it briefly, and put it on the SIT. Push it all the way up until it is stuck.
-Click on the "Acquire data" button at the Acquisition Dashboard. Now there should be dots appearing in your dotplots and histogram. You may want to change the voltage settings at the "Instrument" window (top, middle) so your population of dots appears where you want them.
-Check if the **Threshold Rate** (on top in the Acquisition Dashboard) is below 12,000. If it is higher you can adjust the Flow Rate (lower right corner of the Acquisition Dashboard) to Low or dilute your sample. When the Threshold Rate is low you can set the Flow Rate to High (or concentrate your sample).
-When everything is set correctly (flow rate, instrument settings) you can set the amount of **Events To Record** (default is 10,000, but 50,000 looks better) and click on the Record Data button. Now your data is stored and the flow will stop recording automatically when it has the data of 10,000 (or any other value you set) events.
-Click on the **Remove Tube** button on the Acquisition Dashboard and remove your tube while holding the SIT all the way to the left. Let the SIT go and the needle is cleaned automatically.
-If you want to run another sample click on the **Next Tube** button on the Acquisition Dashboard. A New Tube will appear and you can rename it. First click on the Acquire Data button and check if flow rate and settings are still correct to optimally visualize your data prior to clicking on the Record Data button.

**Cleaning procedure after use**

When you're finished measuring your samples you have to do a short cleaning procedure in order to prevent bacterial growth inside the flow cytometer. There are three tubes, labeled "CLEAN", "RINSE", and "FACS FLOW", in the rack next to the flow cytometer.
-Take tube "**CLEAN**" and place it onto the SIT and run it (clicking Acquire Data on the Acquisition Dashboard) for **one minute** as indicated on the Acquisition Dashboard (upper left corner).
-Click on the Remove Tube button on the Acquisition Dashboard and remove your tube while holding the SIT all the way to the left. Let the SIT go and the needle is cleaned automatically.

-Take tube "**RINSE**" and place it onto the SIT and run it (clicking Acquire Data on the Acquisition Dashboard) for **one minute** as indicated on the Acquisition Dashboard (upper left corner).
-Click on the Remove Tube button on the Acquisition Dashboard and remove your tube while holding the SIT all the way to the left. Let the SIT go and the needle is cleaned automatically.
-Take tube "**FACS FLOW**" and place it onto the SIT and run it (clicking Acquire Data on the Acquisition Dashboard) for **one minute** as indicated on the Acquisition Dashboard (upper left corner).
-Click on the Remove Tube button on the Acquisition Dashboard and remove your tube while holding the SIT all the way to the left. Let the SIT go and the needle is cleaned automatically.

**Flow cytometer shut down**

When you're done measuring your samples and you've cleaned the flow cytometer, you can shut down the flow cytometer and computer.
-First, click on "**Instrument>Fluidics Shutdown**" this will open a small text box where you should confirm the Fluidics Shutdown by clicking OK.
-When the window pops up that the Fluidics Shutdown is complete, you can start the analysis of your samples (can be done at any time) or you can shut down the software (click on X in upper right corner), shut down the computer (start>shut down, lower left corner), and shut down the flow cytometer (push the green button on the left side of the flow cytometer). When the flow cytometer shuts down it will make some noise (depressurizing the system), this is normal.

**Data processing and analysis**

You can use the FACSDiva software for some types of analysis. There are however two major drawbacks: i) the program cannot make overlays (multiple histograms in a single graph), ii) the figures generated can only be exported as .jpg files, which means that the resolution is good enough for PowerPoint presentations or printing but is not sufficient for posters and especially not sufficient for articles. You can use different programs that can be useful (e.g. for making overlays) such as WinMDI, Cyflogic, and FlowJo. -WinMDI (also present on this computer) can make overlays and figures can be exported as scalable vector graphics. It is freeware and can be found at http://facs.scripps.edu/software.html. Cyflogic is very convenient when you want to do multiple comparisons and it looks a bit better than WinMDI. However, the figures cannot be exported as svg. Also Cyflogic is freeware and can be found at www.cyflowgic.com. FlowJo is probably the best program, looks great, can make overlays and scalable graphics. However, we don't have the program because it is not for free (it is actually very expensive). More information about FlowJo can be found at www.flowjo.com.
The data can be collected from this PC and stored on a memory stick or external hard drive for further analysis on another computer. You can do this at any time, e.g. just after you measured or on another day.

-Turn the computer on (you don't have to start the flow cytometer) and start the FACSDiva software as described above.

-Open your personal folder and the desired experiment by clicking on the + in front of your name and double click the desired experiment.
-Right click on your experiment and click on Export>FCS files…
-Click on the OK button of the popped up Export parameter window. Default settings are ok to export and you can open these file and any other flow cytometer analysis software.
-Browse to your memory stick or external hard disk and click on the Save button. All the files in your experiment will be saved with the names of the corresponding tubes in a folder bearing the name of your experiment.

-You may want to do some analyses using the FACSDiva software. You can already do some analyses in between your measurements so that all downstream tubes will already be analyzed during the actual acquisition.
-To analyze your data using the FACSDiva software, there are several useful tools at the "Workbook" (on the right of your screen where you made the dotplots and histograms).
-To make gates (give specific populations within the entire population of your cells a color and have separate statistics), you can click on the $10^{th}$ to $17^{th}$ icon in the worksheet window and select the desired population in a dotplot or histogram. All files in your experiment will show the same gate with the same color.
-To make sense out of your data you need some basic statistics, which you can find by right clicking on a certain dotplot or histogram and click "Create Statistics View".
-You can change the parameters or populations shown by right clicking on the statistics table and clicking on "Edit Statistics View". The statistics are added to your worksheet and are displayed for all tubes in your experiment.

**Changing fluids**
The indicators in the "Instrument" window of the FACSDiva software should not be red! If there are one (or more) red indicators you should change the corresponding fluid or empty the waste container.
-To change "FACS Flow", "FACS Clean", or "Shutdown Solution" you get a new box (in the drawers on the right underneath the centrifuge). Unscrew the cap with the tube and indicator sensor on top of the "empty" box at the cart and place the new box on the same place in the cart. Screw the cap with tube and indicator sensor tightly on the new box.
-Click on "Instrument>Cleaning Modes>"**Prime after Tank Refill…**" and check the box (or boxes when you changed more fluids) of the corresponding fluid you changed. Click on ok and after a few seconds a window will pop up indicating the Tank prime is complete, click on OK.
-Check if there is not too much air trapped inside the filter of the liquid you changed on the back of the fluidics cart. If half the filter is filled with air, unscrew the small white cap on the top of the filter. Screw it back on the filter when the liquid is coming out and there is no more air trapped.

-To empty the waste container, unscrew the cap with tube and indicator sensor. Empty the waste in the sink and put it back on the same place on the cart. Screw the cap with tube and indicator sensor back on the container.
-Click on "Instrument>Cleaning Modes>"**Prime after Tank Refill…**" and check the box of the "**FACS Clean**" fluid. This will put chlorine in the waste container so that

the bacterial cells that come into it will be killed. Click on ok and after a few seconds a window will pop up indicating the Tank prime is complete, click on OK.

**Weekly short cleaning procedure**

Especially when the flow cytometer is not frequently used, it should be cleaned once a week. Normally I will do this, but when I'm absent here you'll find the weekly cleaning procedure.

-Turn the flow cytometer and computer on and start the FACSDiva software as described above.

-Empty the waste container (even if there is not much in there) and check if there is not too much air trapped inside the filters on the back of the fluidics cart, as described above.

-Place the tube with **FACSClean** onto the SIT.

-Click on Instrument>Cleaning Modes>**Bubble Filter Purge** and click on OK on the popped up window.

-Click on Instrument>Cleaning Modes>**Clean Flow Cell…** and click on OK on the popped up window. Subsequently, click on Instrument>Cleaning Modes>**De-gas Flow Cell** and click on OK on the popped up window. Repeat these two steps 3 times.

-Click on Instrument>**Fluidics Startup** and click on OK on the popped up window.

-Take tube "**CLEAN**" and place it onto the SIT and run it (clicking Acquire Data on the Acquisition Dashboard) for **ten minutes** as indicated on the Acquisition Dashboard (upper left corner).

-Click on the Remove Tube button on the Acquisition Dashboard and remove your tube while holding the SIT all the way to the left. Let the SIT go and the needle is cleaned automatically.

-Take tube "**RINSE**" and place it onto the SIT and run it (clicking Acquire Data on the Acquisition Dashboard) for **ten minutes** as indicated on the Acquisition Dashboard (upper left corner).

-Click on the Remove Tube button on the Acquisition Dashboard and remove your tube while holding the SIT all the way to the left. Let the SIT go and the needle is cleaned automatically.

-Take tube "**FACS FLOW**" and place it onto the SIT and run it (clicking Acquire Data on the Acquisition Dashboard) for **ten minutes** as indicated on the Acquisition Dashboard (upper left corner).

-Click on the Remove Tube button on the Acquisition Dashboard and remove your tube while holding the SIT all the way to the left. Let the SIT go and the needle is cleaned automatically.

-First, click on "**Instrument>Fluidics Shutdown**" this will open a small text box where you should confirm the Fluidics Shutdown by clicking OK.

-Shutdown the software, the computer, and the flow cytometer as described above.

**Monthly long cleaning procedure and calibration**

The first Monday of each month the flow cytometer has to be cleaned and calibrated. Normally I will do this, but when I'm absent here you'll find the weekly cleaning procedure.

-Turn the flow cytometer and computer on and start the FACSDiva software as described above.

-Empty the waste container (even if there is not much in there) and check if there is not too much air trapped inside the filters on the back of the fluidics cart, as described above.
-Make sure that there is enough "**FACSClean**" (about half) and "**Shutdown Solution**" (about full).
-Click on Instrument>Cleaning Modes>**Long Clean…** and click on OK on the popped up window. This will take about one and a half hour. After this the normal weekly procedure should be followed. However, before starting the fluidics shutdown the flow cytometer should be calibrated.

-Open a silver sachet from the blue/white box named **BD FACS$^{tm}$ 7-Color Setup Beads**, which is in the fridge.
-Open the tube and add about 1 ml of **BD FACS$^{tm}$ Setup Bead Diluent B**.
-Vortex the tube for at least 10 seconds and place the tube onto the SIT.
-Start the program **BD FACSCanto Software**, by clicking the icon on the left of the desktop, press enter when the software is asking for a password (there's no password required).
-Click on Cytometer>Setup>Standard Setup… and click on Next button.
-Click on Next button when "Run setup in Manual mode" is checked.
-load the tube with the setup beads onto the SIT and click on OK
-Remove the tube when calibration is finished
-The setup report is printed automatically.
-Click on **Finish**
-When you close the program a popup appears with two options i) Run fluidics shutdown and exit ii) Exit only. Select first option and click on OK if you don't want to run samples on the FACS. Select the second option and click on OK if you do want to run samples on the FACS.